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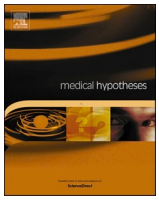

Research Article

'Hypercobalaminuria' – Is urinary cobalamin loss a potential determinant of parenteral cobalamin (B_{12}) efficacy in Pernicious Anaemia?

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ABSTRACT

It is unknown why many patients with pernicious anaemia are satisfactorily treated with injections of hydroxocobalamin or cyanocobalamin every 1–3 months whereas others require far more frequent replacement regimens, sometimes even weekly. A substantial but inconstant fraction of an injected dose of cobalamin is excreted in the urine within 72 h of injection, with subsequent loss of variable smaller amounts. We hypothesize the existence of 'hypercobalaminuria', whereby increased urinary cobalamin losses constitute a currently unrecognized factor influencing treatment refractoriness in some individuals. The hypothesis is testable by comparing cobalamin urinary losses in patients needing frequent as opposed to 1–3-monthly injections of cobalamin to remain symptom free. It implies that 'less-responsive' patients are likely to have significant hypercobalaminuria.

Introduction

Vitamin B_{12} (cobalamin) is an essential nutrient required for haematopoiesis and normal neurological function [1,2]. It is only available from biosynthesis by a few soil, aquatic and rumen microorganisms [3,4]. Their symbiosis with animals enriches foods such as meat, fish, milk, eggs, and dairy products [5].

Deficiency of the vitamin is a significant global health issue affecting all ages. It arises from either dietary insufficiency or malabsorption. Dietary insufficiency commonly occurs in those following an unsupplemented vegetarian or vegan diet [6]. A common cause of malabsorption is pernicious anemia (PA) characterized by autoimmune gastritis (AIG) with destruction of parietal cells and loss of intrinsic factor (IF), which is required for absorption of cobalamin in the terminal ileum [7].

In the UK, maintenance treatment of PA is usually via intramuscular injection of hydroxocobalamin, every 2–3 months, given by a health care professional. In North America (USA and Canada) the preferred form is cyanocobalamin, administered monthly [8].

Regardless of the form and frequency conventionally used, many patients become symptomatic prior to their next scheduled injection appointment [9]. A survey of 'Pernicious Anaemia Society' (PAS) members found that many patients feel they do not receive their injection sufficiently frequently for adequate and continuous symptom relief [10].

The PAS and James Lind Alliance (PAS/JLA) recently identified ten research priorities for PA through a Priority Setting Partnership [11]. Two of these relate to treatment efficacy, 1) why do people with PA need cobalamin injections at different frequencies? and 2) why do some people with PA continue to experience recurrence of symptoms, even after treatment with cobalamin?

The hypothesis

As with any nutrient, deficiency essentially arises from three main sources in genetically normal individuals: inadequate dietary intake, inadequate absorption, increased utilisation and/or losses. However, regarding cobalamin, the latter possibility has been overlooked. Other

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than a few publications in the 1950′s and 60′s [12–14] and the 2000 s-2010 s [15–17] there is a remarkable paucity of literature concerning urinary cobalamin losses.

Total body content of cobalamin amounts to more than 1 mg with an approximate daily loss of 0.12 % [18,19]. In healthy adult individuals this is replaced by a recommended dietary allowance of 2.4 mcg cobalamin.

Patients with PA lose the capacity for uptake of the vitamin and therefore depend upon pharmacological doses. These are usually administered as a 1 mg dose given intramuscularly every one (cyanocobalamin) to three months (hydroxocobalamin). If this dose remained within the body, it would ensure a sufficient supply for more than a year. However, retention of injected cobalamin depends on the form administered as well as the pharmaceutical vehicle employed [20] – in addition to currently unknown individual factors.

Once the injected cobalamin reaches the blood stream any fraction of the vitamin that remains unbound to proteins is rapidly cleared by the kidney and excreted in the urine [21]. Cyanocobalamin binds only to the cobalamin binding proteins transcobalamin and haptocorrin whilst hydroxocobalamin also forms a weak complex with other proteins, notably albumin. This difference explains why cyanocobalamin is cleared faster from the body than is hydroxocobalamin [21].

In addition to clearance of free cobalamin the kidney also plays a role in handling cobalamin bound to transcobalamin and albumin. Transcobalamin, with a molecular weight of 43 kDa, passes into the glomerular filtrate but virtually all is reabsorbed via the multifunctional receptor megalin [22]. Likewise, most of filtered albumin bound cobalamin is reabsorbed, both by megalin and another multifunctional receptor – cubilin [9].

Urinary cobalamin losses vary between individuals, both in the basal state and following a large bolus intramuscular injection [16,17,23–26].

We posit that excess urinary loss of cobalamin ('hypercobalaminuria') may constitute a hitherto unrecognized factor contributing to treatment inefficacy.

A consequence of this phenomenon would be the existence of a subgroup of individuals with PA who, after cobalamin injection therapy, display a more rapid loss of cobalamin due to excessive cobalaminuria ("hypercobalaminuria"). Such individuals would experience a faster return of symptoms of cobalamin deficiency than others and require more frequent injections.

A simple analogy to illustrate this point is to consider two bathtubs, one which cannot be filled due to a defective tap/faucet (individuals with PA) whereas the other *also has a leaky plug* (individuals with PA *and* 'hypercobalaminuria'). In the former situation, the bathtub is easily filled with buckets of water, whereas in the latter the continued excess losses necessitate more frequent water replacement (Fig. 1).

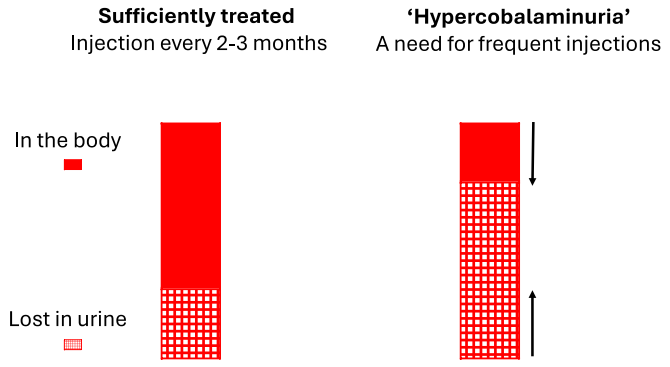

Fig. 1. *Model for distribution of an injected dose of cobalamin.* The figure presents a schematic overview for the distribution of cobalamin following intramuscular injection of 1 mg hydroxocobalamin in patients well treated with a standard injection schedule (left panel) and in patients requesting frequent injections possibly explained by 'hypercobalaminuria' (right panel).

In essence, we propose that two distinct cohorts of PA (AIG-associated cobalamin deficient) individuals may exist, with one cohort exhibiting an additional complication of increased urinary cobalamin losses, resulting in poorer retention of the injected dose. This compounds the absorptive defect, hence necessitating more frequent cobalamin replacement.

Evolution of the hypothesis

Urinary cobalamin excretion depends on the given dose, its mode of administration (oral, parenteral, intramuscular and less commonly subcutaneous injections), its formulation (i.e. cyano- or hydroxocobalamin) and its excipients. Intramuscular injection of 100 μg results in excretion that is detectable for up to 12 h, both in healthy controls and patients with PA [27,28].

Patients with PA display no haematological response to small oral cobalamin doses (i.e. 5 μg) [29,30], while intramuscular injections will achieve symptom remission [31]. Relatively few (6 %) patients with PA receive maintenance therapy with high dose oral cobalamin, but only if there is no neurological involvement [32,33]. However, most patients rely solely on intramuscular injections.

The amount of cobalamin excreted in urine (E) 8 h after an intramuscular injection of doses between 5 and 1000 mcg normally depends on the dose administered (D) according to the following equation: $E = D - 1.2D^{0.89}$ [34]. We do not know to what extent this formula can be reliably used in PA patients. Intramuscular injections of 42.2 μg to 211 μg of cobalamin in either ascending or descending order in healthy controls (n = 6) and PA patients (n = 6) showed maximal urinary excretion at the highest doses with no statistically significant differences between the two groups [12]. Successive daily administration of cobalamin from high to low dose revealed a trend wherein for the lowest dose of 42.2 μg, patients with PA excreted twice as much cobalamin as healthy controls. However, these differences did not reach statistical significance, possibly due to the small cohort size [12].

Several decades of research paucity have obstructed our understanding of the complex relationships between mode of administration, dose, compartmentalisation, excretion patterns and reversal of clinical symptoms in patients with PA. Prior studies on urinary cobalamin excretion are few and far between and only performed on small cohorts. Studies examining urinary cobalamin excretion amongst PA patients with varying needs of intramuscular injection to achieve symptom remission are lacking.

Many patients with PA become symptomatic before their next scheduled injection appointment. However, a lack of recognition of this phenomenon has led to patient dissatisfaction, with some patients completely withdrawing from care seeking [9]. A simple explanatory mechanism would be apposite. If hypercobalaminuria does explain the apparent differences in response to cobalamin replacement it allows for a simple test to identify those in need of more frequent treatment. It would have important implications for rational modification of the current recommended guidelines for the treatment of PA.

Obviously, proof of this hypothesis raises the next question: 'what drives aberrant cobalamin excretion?' The most likely explanation would be increased clearance of free cobalamin. While cyanocobalamin is freely cleared, provided all binding capacity of transcobalamin and haptocorrin is saturated [35], hydroxocobalamin is partly retained bound weakly to other proteins, mainly albumin.

A less likely explanation for increased excretion would be an altered function of the receptor ensuring the reuptake of any filtered cobalamin bound to transcobalamin. Both options are testable. Transcobalamin can be measured in the urine, and the type of cobalamin excreted in the urine can be identified.

Hypothesis testing

The hypothesis could be tested by comparing urinary cobalamin

excretion in patients with PA treated with injections of cobalamin (either hydroxocobalamin or cyanocobalamin) and relating it to the requested interval needed to significantly alleviate symptoms. 24-h urinary samples could be collected for the first three days post-injection. In addition, blood samples could be taken at baseline and daily for the following three days. Cobalamin could be measured in urine and plasma by standard techniques (chemiluminescent or microbiological assay).

Urine samples would need to be collected from clinically well-characterised individuals, ideally within a primary care population. It would be important to avoid bacterial contamination of sample collection to ensure that microbial synthesis or consumption of cobalamin does not influence the measurements. Collecting 24-hour urine samples for several days from many participants can be cumbersome and may lead to compliance issues or incomplete data, though these difficulties are not insurmountable with careful planning and supervision. However, collection of 24-h urine is important because urinary excretion of cobalamin is dependent on urine volume [15].

Measurement of cobalamin in blood samples will help to judge a relationship between this value and urinary excretion and thereby possibly ease future identification of individuals with a high cobalamin clearance. However, in interpreting plasma cobalamin levels it should be remembered that it comprises the sum of the freely filtered unbound vitamin, the vitamin bound to haptocorrin (slow turnover, not filtered in urine), holo-transcobalamin (fast turnover, partly filtered in the urine and reabsorbed in the kidney) and the vitamin bound to albumin (partly filtered and reabsorbed in the kidney) (15, 22).

Implications

The importance of the hypothesis is suggested by the recently identified PAS/JLA research priorities (see above). If hypercobalaminuria does explain the apparent differences in response to cobalamin replacement it allows for a simple test to identify those in need of more frequent treatment. Hence it would have important implications for rational modification of the current recommended guidelines for the treatment of PA.

If our hypothesis proved incorrect it would be necessary to consider alternative explanations for interindividual variations in treatment responses. For example, some individuals might require a higher level of cobalamin to ensure adequate cellular uptake and/or utilisation, perhaps due to polymorphisms in transcobalamin and/or its cellular receptor (CD320) and/or cobalamin dependent enzymes [36]. It is also possible that the presence or absence of antibodies to the CD320 receptor might influence the required injection frequency [37].

Another possibility is that patients with PA receiving life-long therapy with high-dose cobalamin (orally or in combination with intramuscular injections [32]) either innately possess, or undergo, gut microbiome transformations; cobalamin is known to regulate gut microbial ecology [38]. This might alter the abundance of plasma cobalamin analogues such that ‘authentic’ cobalamin uptake via the CD320 receptor is impaired. This has yet to be investigated but could explain why some PA patients require more frequent injections. The association between neurologic abnormalities and a greater abundance of plasma analogues in cobalamin deficient PA patients [39] lends some credence to this alternative hypothesis.

Conclusion

The possibility that increased urinary cobalamin loss can contribute to deficiency and its associated symptoms has been hitherto neglected. Such a mechanism affords a potential explanation for poor ‘treatment responses’ in a subset of patients with PA. The hypothesis is testable by measuring total urinary excretion of cobalamin for several days following its parenteral administration to well characterised deficient individuals and determining the relationship of the rate of loss to plasma levels of cobalamin and duration of symptom relief.

It would also be possible to gain insight into the likely underlying mechanism for any increased excretion, by determining whether the loss in such individuals primarily comprises only free or also transcobalamin bound cobalamin, the former being more likely.

Consent statement/Ethical approval

Not required.

CRediT authorship contribution statement

Andrew McCaddon: Writing – original draft, Conceptualization. **Ebba Nexo:** Writing – review & editing. **Ralph Green:** Writing – review & editing. **Kourosh R Ahmadi:** Writing – review & editing. **Luciana Hannibal:** Writing – review & editing. **Alfie Thain:** Writing – review & editing. **Joshua W Miller:** Writing – review & editing.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] Oh R, Brown DL. Vitamin B12 deficiency. Am Fam Phys 2003;67:979–86.
[2] Wolffenbuttel BH, Owen PJ, Ward M, Green R. Vitamin B(12). BMJ 2023;383:e071725.
[3] Martens JH, Barg H, Warren MJ, Jahn D. Microbial production of vitamin B12. Appl Microbiol Biotechnol 2002;58:275–85.
[4] Croft MT, Lawrence AD, Raux-Deery E, Warren MJ, Smith AG. Algae acquire vitamin B12 through a symbiotic relationship with bacteria. Nature 2005;438:90–3.
[5] Watanabe F, Bito T. Vitamin B(12) sources and microbial interaction. Exp Biol Med (Maywood) 2018;243:148–58.
[6] Niklewicz A, Smith AD, Smith A, Holzer A, Klein A, McCaddon A, et al. The importance of vitamin B(12) for individuals choosing plant-based diets. Eur J Nutr 2022.
[7] Green R, Allen LH, Bjorke-Monsen AL, Brito A, Gueant JL, Miller JW, et al. Vitamin B12 deficiency. Nat Rev Dis Primers 2017;3:17040.
[8] Green R. Vitamin B(12) deficiency from the perspective of a practicing hematologist. Blood 2017;129:2603–11.
[9] Cotton M, McCaddon A. Examining the diagnosis and treatment experiences of people living with autoimmune gastritis and pernicious anemia. J Patient Exp 2023;10:23743735231151767.
[10] Hooper M, Hudson P, Porter F, McCaddon A. Patient journeys: diagnosis and treatment of pernicious anaemia. Br J Nurs 2014;23:376–81.
[11] Staley K, Ahmadi KR, Carter K, Cowan K, Seage H, Visser P, et al. Research priorities in pernicious anaemia: James Lind Alliance Priority Setting Partnership. BMJ Open 2022;12:e065166.
[12] Sokoloff MF, Sanneman Jr EH, Beard MF. Urinary excretion of vitamin B12. Blood 1952;7:243–50.
[13] Mollin DL, Ross GI. Vitamin B12 concentrations of serum and urine in the first 72 hours after intramuscular injections of the vitamin. J Clin Pathol 1953;6:54–61.
[14] Adams JF. The urinary excretion and tissue retention of cyanocobalamin by subjects given repeated parenteral doses. J Clin Pathol 1964;17:31–8.
[15] Fukuwatari T, Sugimoto E, Tsuji T, Hirose J, Fukui T, Shibata K. Urinary excretion of vitamin B12 depends on urine volume in Japanese female university students and elderly. Nutr Res 2009;29:839–45.
[16] Shibata K, Hirose J, Fukuwatari T. Relationship between urinary concentrations of nine water-soluble vitamins and their vitamin intakes in Japanese adult males. Nutr Metab Insights 2014;7:61–75.

[17] Tsuji T, Fukuwatari T, Sasaki S, Shibata K. Twenty-four-hour urinary water-soluble vitamin levels correlate with their intakes in free-living Japanese university students. Eur J Clin Nutr 2010;64:800–7.
[18] Bessent RG, Watson WS, MacDonald CM, Adams JF. Application of the occupancy principle in studies of the metabolism of vitamin B12 in man. Clin Sci (Lond) 1980; 58:169–71.
[19] Adams JF, Tankel HI, MacEwan F. Estimation of the total body vitamin B12 in the live subject. Clin Sci 1970;39:107–13.
[20] Buhl E, Buhl BB, Kornerup LS, Nexo E. Vitamin B12 deficiency. Ugeskr Laeger 2024;186.
[21] Breckenridge A, Metcalfe-Gibson A. Methods of measuring glomerular-filtration rate: a comparison of inulin, vitamin-B 12 and creatinine clearances. Lancet 1965; 2:265–7.
[22] Nielsen R, Christensen EI, Birn H. Megalin and cubilin in proximal tubule protein reabsorption: from experimental models to human disease. Kidney Int 2016;89: 58–67.
[23] Withey JL, Kilpatrick GS. Hydroxocobalamin and cyanocobalamin in addisonian anaemia. Lancet 1964;1:16–8.
[24] Tudhope GR, Swan HT, Spray GH. Patient variation in pernicious anaemia, as shown in a clinical trial of cyanocobalamin, hydroxocobalamin and cyanocobalamin–zinc tannate. Br J Haematol 1967;13:216–28.
[25] Killander A, Schilling RF. Studies on hydroxocobalamin. I. Excretion and retention of massive doses in control subjects. J Lab Clin Med 1961;57:553–61.
[26] Carkeet C, Dueker SR, Lango J, Buchholz BA, Miller JW, Green R, et al. Human vitamin B12 absorption measurement by accelerator mass spectrometry using specifically labeled (14)C-cobalamin. Proc Natl Acad Sci U S A 2006;103:5694–9.
[27] Chow BF, Lang CA, Davis R, Conley CL, Ellicott CE. The appearance of vitamin B12 activity in urine after oral and intramuscular administration to man. Bull Johns Hopkins Hosp 1950;87:156–63.
[28] Conley CL, Krevans JR, Chow BF, Barrows C, Lang CA. Observations on the absorption, utilization, and excretion of vitamin B12. J Lab Clin Med 1951;38: 84–94.
[29] Hall BE, Morgan EH, Campbell DC. Oral administration of vitamin B12 in pernicious anemia; presence of intrinsic factor in Berkefeld-filtered polled human gastric juice; preliminary report. Proc Staff Meet Mayo Clin 1949;24:99–107.
[30] Meyer LM, Sawitsky A, Cohen BS, Krim M, Fadem R. Oral treatment of pernicious anemia with vitamin B12. Am J Med Sci 1950;220:604–9.
[31] Unglaub WG, Rosenthal HL, Goldsmith GA. Studies of vitamin B12 in serum and urine following oral and parenteral administration. J Lab Clin Med 1954;43: 143–56.
[32] Graham ID, Jette N, Tetroe J, Robinson N, Milne S, Mitchell SL. Oral cobalamin remains medicine's best kept secret. Arch Gerontol Geriatr 2007;44:49–59.
[33] Andres E, Serraj K. Optimal management of pernicious anemia. J Blood Med 2012; 3:97–103.
[34] Chesterman DC, Cuthbertson WF, Pegler HF. Vitamin B12; excretion studies. Biochem J 1951;48:LI–LII.
[35] Slapak M, Hume DM. A new method of estimating glomerular-filtration rate: use of diminishing blood concentration of labelled vitamin B12. Lancet 1965;1:1095–7.
[36] McCaddon A, Carr DF, Peter H, Moat SJ, Quadros EV. Transcobalamin receptor gene polymorphisms and mutation in an elderly population. Clin Nutr ESPEN 2023;55:425–7.
[37] Pluvinage JV, Ngo T, Fouassier C, McDonagh M, Holmes BB, Bartley CM, et al. Transcobalamin receptor antibodies in autoimmune vitamin B12 central deficiency. Sci Transl Med 2024;16:eadl3758.
[38] Degnan PH, Taga ME, Goodman AL. Vitamin B12 as a modulator of gut microbial ecology. Cell Metab 2014;20:769–78.
[39] Carmel R, Karnaze DS, Weiner JM. Neurologic abnormalities in cobalamin deficiency are associated with higher cobalamin "analogue" values than are hematologic abnormalities. J Lab ClinMed 1988;111:57–62.